IONIC AND WATER BALANCE OF PLANARIANS[1]

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Planarians, order Tricladida, class Turbellaria, are flatworms, mostly free-living, that have invaded fresh water, the oceans and land. All have the same general morphology and, except for some of the land planarians, are relatively small, usually not exceeding a centimeter or so in length. All are unarmored and most of them are ciliated. All have a flattened, leaflike body and a much branched intestine with a single external orifice (Hyman, 1951).

Little is known about the mechanisms whereby these organisms can adjust and maintain water and ionic content in such varying habitats. Information that is available is, except for a small amount of very recent work, summarized excellently by Hyman (1951). While some variations in the excretory system have been noted between marine and fresh-water forms, most of our physiological knowledge consists of demonstrations that planarians are influenced by their osmotic environment, swelling or shrinking appropriately as the medium is changed (Adolph, 1925). Some planarians are euryhaline and one (*Gunda = Procerodes*) has been studied by Pantin (1931) and by Beadle (*cf.* Beadle, 1934). A discussion of this work is to be found in Krogh's book (Krogh, 1939). The present report gives certain basic information about water and ionic contents of marine and fresh-water planaria, together with preliminary studies of fluxes of the materials into or out of the animal body. The animals were chosen, in large part, because of their availability. The common laboratory planarian, *Dugesia tigrinum,* was used as the fresh-water form, the ectoparasite of *Limulus, Bdelloura* (species not determined) for the marine animal. Regrettably, *Gunda* was not available at the time these studies were carried out.

MATERIALS AND METHODS

Dugesia are maintained in pond water stock culture in this laboratory. They are fed frequently on raw liver and are fine, healthy animals relapsing now and then into a sexual phase. The cultures are usually propagated by cutting the animals in half transversely and then allowing regeneration to take place.

Bdelloura were obtained from *Limulus* collected on Cape Cod. The worms are easy to obtain and live well in sea water. While closely resembling *Dugesia* in general internal structure, size and shape, behavior is quite different. Compared to *Dugesia* they are very active, looping along with quiet vigor by means of the adhesive organs. *Dugesia*-like gliding is rarely seen. So far, attempts to feed *Bdelloura* have failed. Similarly, attempts to demonstrate chemical attraction to various portions of *Limulus* have failed in spite of the fact that *Bdelloura* is reported to exist only on *Limulus*.

Both types of worms were transferred, as desired, by pipettes or small spatulae. To obtain wet weights, animals were drained on fine fibered filter paper and then transferred to tared strips of Parafilm for rapid weighing on a torsion balance.

Na and K were determined by flame photometry on extracts prepared by heating worms (20–60-mg. samples) in 10 ml. of H_2O with 3 to 4 drops of glacial acetic acid added. Nitric acid digests of residues from the hot acid water extraction showed that essentially complete removal of the ions had taken place. Chlorides were determined on the same extracts by means of a Cotlove type chloride meter.

Worms were labeled with Na^{24}, K^{42} and Cl^{36} by standard methods.

In general, influxes of the ions were determined by counting radioactivity in individual worms placed on a waxed plate directly under a thin-window Geiger counter tube. Levels of activity were chosen such that one-minute counts would suffice, the same worm being assayed at appropriate intervals. Effluxes of the ions were determined by essentially the same procedure, zero time being the time

TABLE I

Summary of data for Dugesia and Bdelloura taken from normal cultures or sea water. Concentrations as mM/Kg. Inulin values determined by C^{14} labelled inulin in trace amounts, calculated as $\dfrac{counts/Kg\ animal}{counts/Kg\ medium}$. Numbers in parentheses indicate number of determinations averaged.

	Na	Cl	K	Inulin value	% Dry
Dugesia (22) Normal Dugesia medium* Dugesia, (2) (in high salt)	14.6 ± 0.5 0.2 20	18.8 ± 0.7 0.3 28	38.2 ± 0.7 0.02 27	2.0	22
Bdelloura (20) Sea water	122 ± 1.4 440	135 ± 2.1 510	97 ± 0.3 10	5.7	25

* Concentration varied, in general between limits for Na, 0.8-0.1 mM. High salt medium for Dugesia = NaCl 10 mM, KCl 10 mM in pond water.

of immersion of the animals in the non-radioactive solution (10^2 to 10^3 times the volume of the worm) following a labelling period in radioactive solution. Tritiated water efflux was determined on worms labelled overnight in tritiated water by transferring them rapidly with stirring to 1-ml. volumes of non-tritiated medium. Aliquots of the washing media were then counted in the liquid scintillator. "Inulin spaces" were determined by adding C^{14}-labeled inulin in a few instances, dry aliquots of extracts being counted in a gas flow counter.

RESULTS

Ionic and water contents

Table I summarizes the analytical data for both worms. As is usual in comparing marine and fresh-water forms, Na and Cl concentrations are quite different, whereas potassium concentration varies less. *Dugesia* concentrates all three ions, *Bdelloura* concentrates K but maintains low concentrations of Na and Cl as compared to the environment.

Some attempts have been made to acclimatize *Dugesia* to high salt concentrations with little success as yet. As soon as the salt concentration of the medium begins to approach that of the animals, they appear very unhappy and stop feeding. On the other hand, worms kept for two weeks in a 20 mM mixture of NaCl and KCl in pond water, during which time they did not feed, recovered and enjoyed a good liver meal within 24 hours after being placed back into normal pond water medium. Table I includes analytical data on the average of two samples of animals from the high salt culture. K decreases by about 30% as compared to controls in spite of a 500-fold increase in external K. Na and Cl contents of the animals increase nearly 50%, the external concentrations having gone up about 100 times. Clearly the internal ionic composition of the worms is not highly dependent on the ionic composition of the medium. A variety of studies has been

TABLE II

Final relative weights and ionic contents of Bdelloura maintained at least two days in sea water diluted as indicated. A series, average of four analyses, B series, average of two analyses. Concentrations as mM/Kg final wet weight or mM/liter of medium.

		Na_i	$\dfrac{Na_i}{Na_o}$	Cl_i	$\dfrac{Cl_i}{Cl_o}$	K_i	$\dfrac{K_i}{K_o}$	Relative weight
100% sea water		134	0.30	135	0.27	97	9.7	100
75% sea water	A	98	0.30	108	0.28	87	11.6	114
	B	75	0.23	96	0.24	80	8.0	118
50% sea water	A	52	0.24	62	0.25	65	12.0	151
	B	56	0.25	67	0.27	64	6.4	165
40% sea water	A	51	0.29	61	0.20	52	13.0	189
	B	61	0.34	61	0.28	52	5.2	210
30% sea water	A	30	0.23	36	0.23	45	15.0	213
	B	35	0.26	51	0.31	45	4.5	221

made on starved worms, worms kept at low temperature, worms cut and allowed to regenerate and even worms maintained in distilled water for several hours. The results show a most remarkable ability of *Dugesia* to maintain constant internal water and salt concentrations under all these conditions.

Murray (1927), in a classic study, notes that a medium of osmolarity of about 15 mM NaCl is optimum for growth of *Dugesia* explants in tissue culture.

Bdelloura can live for two weeks or more in diluted sea water. In concentrations less than 50% sea water, however, the worms move only sluggishly and look as though they are in poor condition. In dilute sea water, the worms increase in body weight and show no signs of return to original size while in the experimental medium. Normal weight is regained on return to normal sea water. Table II gives data on weight changes and body concentrations of Na, Cl and K in worms held two or more days in sea water diluted either with distilled water (A) or 10 mM KCl (B) in order to hold the K level of the environment approximately

constant.   Na and Cl concentrations of the worms adjust to the various dilutions as indicated by relatively constant inside-to-outside ratios.   K concentrations of the animals decrease in proportion to the increase in body weight due to swelling in dilute media, tending to maintain a constant amount of K per gram original weight, regardless of the external concentration of K or of total salt.

Flux rates of ions and water

With the use of radioactive tracers, K^{42}, Na^{24}, Cl^{36} and tritiated water, flux rates have been determined for *Dugesia* and *Bdelloura*.   These results are reported
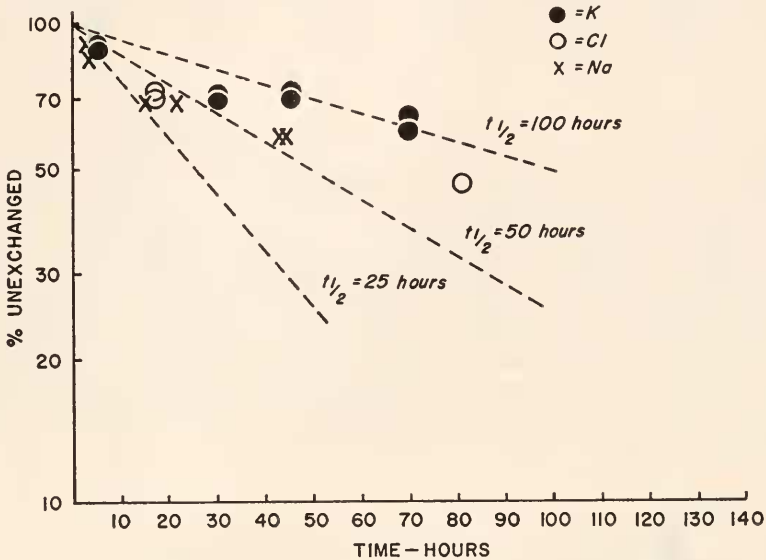

FIGURE 1.   Influx of K^{42} (circles), Na^{24} (dots) and Cl^{36} (crosses), added in trace amounts to normal culture medium, into *Dugesia*.   Results plotted as % unexchanged ion of the worms against time of immersion in radioactive solution.   Straight lines, representing different $t_{\frac{1}{2}}$ values as indicated, are drawn merely to show the variations in slopes of lines, not to fit the experimentally determined values.

in preliminary form since the detailed treatment would require a considerably greater number of observations and, hopefully, a more detailed understanding of the physiological mechanisms involved.

Figures 1, 2 and 3 give results for ion fluxes, on semi log paper, as % unexchanged ion in the worm plotted against time.

Influx of ions into *Dugesia* is very slow following an initial rapid entry (Fig. 1) of unknown significance.   The initial rapid phase amounts to over 10% whereas inulin spaces are much less.   Therefore, more than the "drainage compartment" is involved.   The slow influx, roughly exponential with time, has a $t_{\frac{1}{2}}$ value of over 100 hours for all ions tested.   Because of this very slow labelling, very few efflux rates were determined.   Scattered results, however, indicated that efflux was at least as slow as influx for each ion.

Raising the external K content did increase influx of K^{42} (Table III).   How-

TABLE III

Per cent specific activity of K^{42} in Dugesia after equilibration with K^{42} solutions of ionic composition indicated for five hours. Concentrations in mM/Kg. Concentrations measured for solution #1, calculated for the others. The worms in solutions 3 and 4 showed definite signs of deterioration.

	Medium, mM			Planaria			K^{42} Spec. Act.
	Na	Cl	K	Na	Cl	K	
1.	2.4	3.0	1.0	13.3	17	37	6.5
2.	12.0	23.0	11.0	16.4	20	39	8.3
3.	22.0	33.0	21.0	18.7	23	41	13.0
4.	52.0	103.0	51.0	20.4	31	51	21.0

ever, the increase in influx was slight as compared to the external concentration change.

Influx of Na^{24} into *Bdelloura* is complicated (Fig. 2). There appears to be an "unexchangeable" portion of Na in the worms but results are variable with respect to this phenomenon and further investigations are needed.

K^{42} influx into *Bdelloura,* following the usual initial rapid phase, seems simple and uncomplicated, the $t_{\frac{1}{2}}$ value being about 3% of that for *Dugesia*. Since *Bdelloura* and *Dugesia* are similar in size and shape, $1/t_{\frac{1}{2}}$ values for influx, divided by the respective external concentrations, could give a measure of relative permeabilities. In view of the data in Table III, this procedure is questionable but emphasizes that the marine form is considerably more permeable to ions than is the fresh-water form.
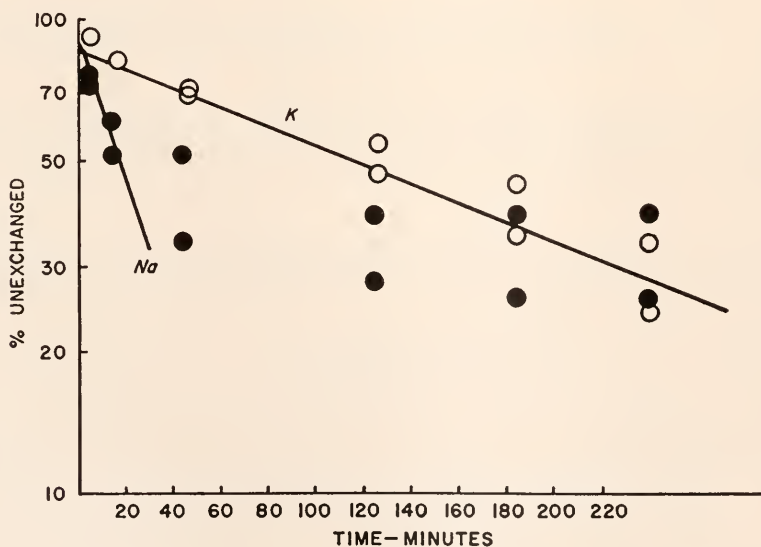

FIGURE 2. Influx of Na^{24} (dots) and K^{42} (circles) into *Bdelloura*. Radioactive element added in trace amounts to normal sea water. Results plotted as in Figure 1.

Effluxes of Na^{24} and K^{42} from previously labelled *Bdelloura* are shown in Figure 3. $T_{\frac{1}{2}}$ values for efflux are essentially comparable to those for influx of the respective ions. As with the influx measurements, K^{42} movement seems relatively uncomplicated, there are again indications of an inexchangeable, or very slowly exchangeable Na fraction.

Tritiated water exchanges very rapidly. The two cases graphed (Fig. 4) represent the smallest (10 mg.) and the largest (55 mg.) *Bdelloura* used. Since body surface is related to weight (see later discussion) it is not surprising that rates of water outflux, measured as half-times of exchange, divided by weights give nearly identical values. For animals of comparable size, efflux rates into 50% sea water following labelling in 50% sea water are identical with those into normal sea water.
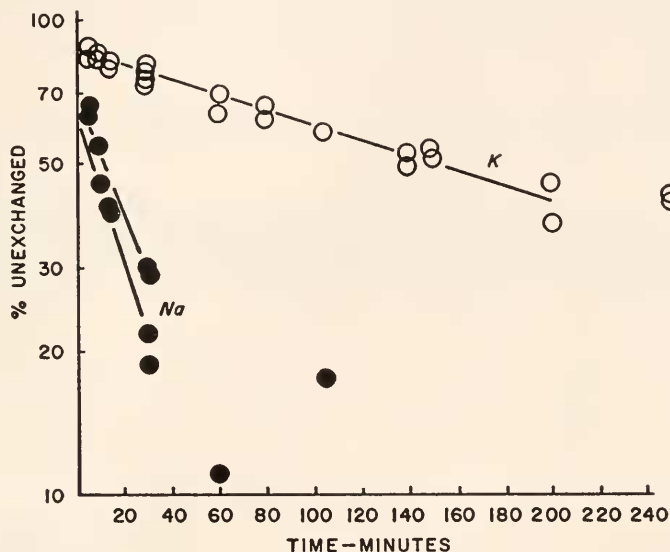

FIGURE 3. Efflux of Na^{24} (dots) and K^{42} (circles) from *Bdelloura* to normal sea water. Worms labelled overnight in sea water with trace concentrations of radioactive ions. Results plotted as in Figure 1.

Similar measurements on influx of tritiated water into *Dugesia* yielded $t_{\frac{1}{2}}$ values of about 160 seconds. Since these animals weighed around 10 mg. the results indicate a somewhat slower rate of water movement in the fresh-water animal.

It should be noted that the $t_{\frac{1}{2}}$ values read from curves such as those of Figure 4 give information on flux rates and are not to be treated as indicators of permeability except to show a very rapid exchange and probably a real or potential rapid net change. Nonetheless both marine and fresh-water forms can be characterized as freely permeable to water and there is certainly no indication that adaption to fresh-water existence has involved the development of a high resistance to the passage of water.

DISCUSSION

The pattern of electrolyte distribution in the two flatworms studied has some interesting general features illustrated by Figure 5. The data on ionic contents
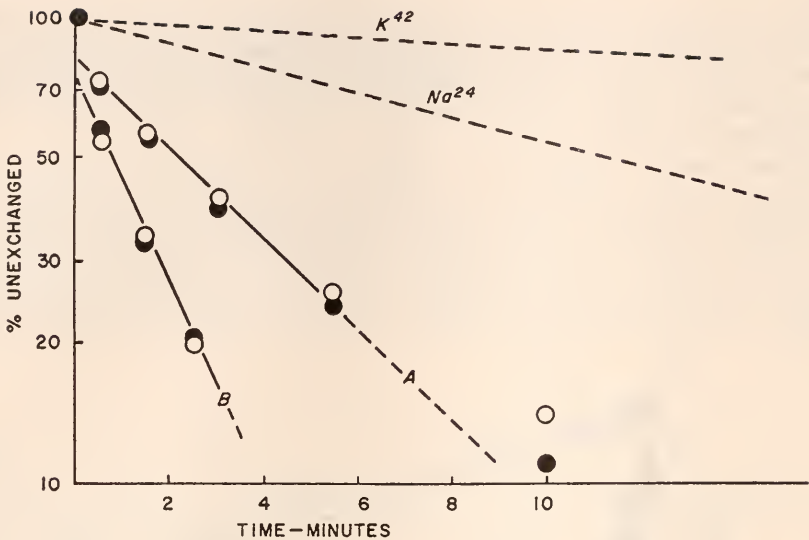
FIGURE 4. Efflux of tritiated water from *Bdelloura*. Animals were equilibrated overnight in tritiated sea water. Curve A, animals in the 55-mg. weight range. Dots: entire procedure in normal sea water, circles: in 50% sea water. Curve B, same as curve A except animals in 10-mg. weight range. K and Na lines from Figure 3.
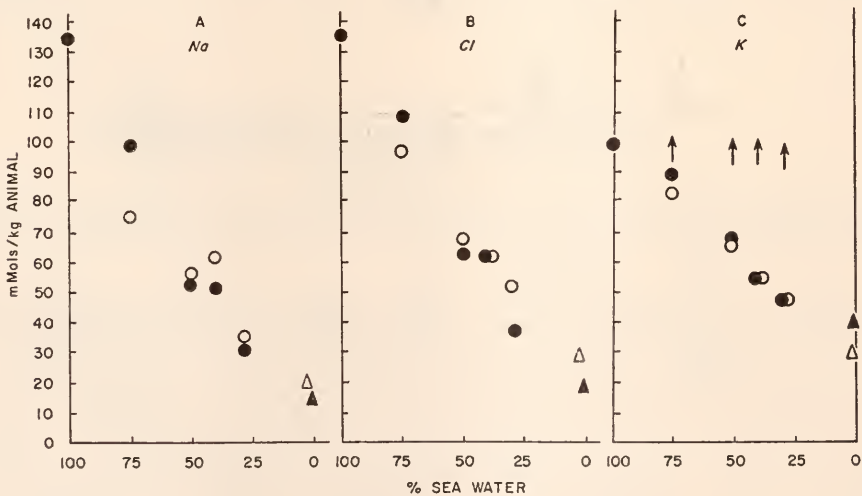

FIGURE 5. Ionic concentrations in *Bdelloura* in different concentrations of sea water as shown in Table II. Concentrations in animals as mM/Kg. final wet weight; in sea water as % sea water. Dilutions of sea water either with distilled water (dots) or 10 mM KCl (circles). In C, arrows point to K concentrations in animals calculated on basis of original, rather than final wet weights. Triangles represent ionic concentrations in *Dugesia* from Table I. Solid symbols, animals in normal medium; open symbols, animals in high salt medium.

for *Bdelloura* given in Table II are plotted against the relative concentration of sea water. The Na, Cl, and K contents of *Dugesia* in normal and high salt media (Table I) have been added to the figure. As previously noted, *Bdelloura* in dilute sea water swell to a new volume which is then maintained, thus indicating that they do not acclimatize in the sense of adjusting their weights (and presumably internal concentrations) back towards normal. The increase in body volume is such that, while K concentration decreases, the total amount of K per animal does not change. Inspection of Figure 5c shows that, in a sense, *Dugesia* is a dilute marine form

TABLE IV

Flux rates of Na, Cl, K and THO (or H_2O) estimated from data in this paper and from other sources as indicated. The values should be taken to indicate order of magnitude only. Influx values unless otherwise noted.

	pM/cm.²/sec.			ml./cm.²/sec. $\times 10^{-5}$
	Na	Cl	K	H_2O
Bdelloura. Efflux to sea water	14.5		1.26	11
Dugesia. (pond water)	0.006	0.006	0.015	<11
Frog.[a] (pond water)	13.8			1
Isolated frog skin.[b] (Ringers)	280			11
Erythrocytes.[c] (Plasma)	0.15		0.026	570
Ascites cells.[d] (Plasma)	3.0		3.0	
Halycystis[e]	10.0	426		
Frog Muscle.[f] (Ringers)	4		4.1	

[a] Jorgensen, 1949.
[b] Ussing, 1955.
[c] Solomon, 1952, and Paganelli and Solomon, 1957.
[d] Hempling, 1958.
[e] Blount and Levedahl, 1960.
[f] Mullins, 1959.

with respect to K. Na and Cl contents of *Bdelloura* decrease more rapidly than K, the internal concentrations being an almost constant fraction of the external. *Dugesia,* however, concentrate both ions in excess over the environment. It is possible that the sodium extrusion mechanisms of the marine form are converted to, or displaced by, a sodium uptake mechanism in *Dugesia.*

In his pioneering studies of *Gunda,* using conductivity methods, Pantin (1931) found that normal animals in sea water had an internal electrolyte concentration equal to about 0.3 M NaCl; animals acclimatized to tap water, a concentration of 0.036 M. It is, of course, impossible to assign specific ionic values but the general

change is in the same range indicated by the comparison of *Dugesia* and *Bdelloura* in Figure 5.

At present it would be premature to calculate any precise values for cellular concentrations. Inulin spaces are so small that they almost certainly indicate only non-draining external fluids rather than intra-animal extracellular spaces. Histological sections of the worms are of little help, except to show that the mesenchyme regions are indeed loosely organized. The points for Na or Cl of Figure 5 lie near a line whose gradient could be interpreted as indicating a "space" of about 25%. This, in turn, would yield a "non-chloride space" K concentration for *Bdelloura* of 130 m*M* and for *Dugesia* of 51 m*M*.

Table IV gives some calculated flux values for *Bdelloura* and *Dugesia,* together with representative values for other forms. Calculation of flux rates has been on the basis of half time exchange values, as illustrated in Figures 1–4, and estimates of body surface. Body surface areas for both types of worms were estimated by tracing outlines of the animals on graph paper, cutting out and weighing the tracings and comparing them with weights of standard areas of the same paper stock. Especially in the case of *Bdelloura,* these values must be regarded as approximate. In normal resting state, *Bdelloura* body surface obviously is not smooth. Both worms can be greatly flattened without losing weight. Hence the surface must be extensible. Even casual inspection of active worms indicates changes in ratios of length/width, width/thickness and so forth. For worms (*Dugesia* and *Bdelloura*) in the weight range of 7–30 mg., the points for surface areas, as derived from weights of paper outlines, plotted against weight of worms, fell fairly well along a straight line indicating 3.5 mm.[2] for each mg. body weight of worm (see Clark and Cowey, 1958, for a discussion of weight-surface relationship in flatworms). This figure has been used in calculating the flux rates for both worms.

No marked differences between influx and outflux values were noted for either worm nor, with *Bdelloura,* was there much effect on either influx or outflux of Na, K or THO of changing the external medium from sea water to 50% sea water. With *Dugesia,* increasing the external K concentration did increase the rate of K[42] entrance but the increase was small compared to the increase in external concentration. The general implication thus is that the movement of the ions into and out of the flatworm is dependent on the properties of the worm and not on the magnitude of a diffusion gradient. The ions measured do not appear to be diffusing along free pathways.

Ions pass in and out at a greater rate in the marine worms than in the fresh-water *Dugesia.* Even if approximate permeability constants are calculated, by dividing flux rates by concentrations, the marine forms are ten or more times more permeable to the ions measured. In *Bdelloura,* sodium influx is higher than potassium influx. In this case, however, conversion of flux rates to relative permeabilities (14.5/440 for Na and 1.26/10 for K) shows the customary lower "permeability" for Na than for K. However, as noted, influx of Na[24] is about the same from either 50% sea water or normal sea water.

In colonizing the marine and fresh-water environments, the flatworms have been faced with severe osmotic and ionic problems. Fresh-water forms are more dilute, have developed mechanisms for uptake of Na and Cl in addition to a K-concentrating system and must possess a most remarkable system for removing excess water. The

results presented here certainly do not indicate the development of an impermeability to water nor, in the light of the low ionic flux rates, is it likely that a filtration resorption system in an excretory device will be found to be the mechanism used. The salt water flatworm may be assumed to be in water equilibrium with its environment, "deficits" due to low ionic contents being made up by organic solutes. On the other hand, *Bdelloura* appears reasonably permeable to ions and yet maintains low body Na and Cl and high K as compared to the environment. Thus a major problem of the salt water form must be the extrusion of cations, a problem found in all cells of higher animals and of marine forms studied so far.

There is, unfortunately, no evidence to show whether ion uptake by fresh-water forms is mediated by the same system, working in reverse, that accounts for ion extrusion in marine forms. Similarly, one cannot tell whether ion movements are linked to water movements, especially in the fresh-water forms.

Summary

1. The Na, K and Cl concentrations (mM/Kg. wet weight) of *Dugesia tigrinum* are 14.6, 38.2 and 18.8. For the marine triclad *Bdelloura* the concentrations are, respectively, 122, 97 and 135.

2. Flux rates for the ions and for tritiated water are faster in the marine flatworms. Exchange of tritiated water, however, is very fast (half time values < 3 minutes) for both forms.

3. *Bdelloura* can live for extended periods of time in dilute sea water, swelling to a new volume which is maintained until returned to a normal sea water medium. *Bdelloura* swollen in dilute sea water do not lose K, even though the K concentration drops due to the volume increase.

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